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# GENETIC STUDIES OF CONGENITAL UPPER LIMB ANOMALIES

AKADEMISK AVHANDLING

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## ABSTRACT

The overall aim of the work reported in this thesis is to improve epidemiological and genetic knowledge of congenital upper limb anomalies (CULA). To accomplish the specific aims of each paper several different methods and approaches have been used such as register studies, array comparative genome hybridization, Sanger sequencing, exome sequencing and functional studies using LacZ enhancer assay, Zebrafish morpholinos and computational transcription factor binding site prediction.

By screening all available medical records and X-rays retrospectively at all hospitals treating CULA in Stockholm county between 1997 and 2007 we identified 562 individuals with CULA resulting in an incidence of 21.5 per 10000 live births. In 99 of the 562 individuals (18%) there was a known occurrence of limb anomalies among relatives. One hundred and thirty of the 562 children (23%) had associated non-hand anomalies. A general table with all studied data that provides good counselling information about gender, laterality, associated anomalies and occurrence among relatives for each type of CULA was created.

The conserved Zone of polarizing Activity Regulating sequence (ZRS) restricts Sonic hedgehog expression to the posterior limb bud and thereby controls anterioposterior patterning in the upper limb. Sanger sequencing of the ZRS in a family with autosomal dominant inherited triphalangeal thumbs with an extra hypoplastic radial thumb revealed an insertion of 13 base pairs segregating with the phenotype. The insertion was predicted to add binding sites for several limb related transcription factors and in a Lac-Z enhancer assay, the insertion mimics the ectopic anterior expression of Sonic hedgehog in the limb bud previously reported to cause radial polydactyly.

A multicentre retrospective study was performed, based on six individuals with overlapping microdeletions of 17q22 including the *NOG* gene. Phenotypic and genotypic comparison between the six included individuals revealed a novel, previously not described 17q22 microdeletion syndrome with symptoms comprising common facial characteristics, multiple bone and joint problems including symphalangism, urogenital malformations and intellectual disability. In addition, some important differences were noted between the individuals, such as hypogonadotrophic hypogonadism and absence of uterus in one individual with a larger heterozygous deletion including *TRIM25*, also named EFP (estrogen-responsive finger protein) Mice carrying a loss of function mutation in one of the *Efp* genes have underdeveloped uterus suggesting that *Efp* could be involved in the normal estrogen-induced cell proliferation of uterus and the uterine swelling

Exome sequencing and Sanger sequencing in three unrelated families with the recently described condition X-linked recessive fusion of the fourth and fifth metacarpals, MF4 (OMIM309630), showed three novel variants in *FGF16*. In one of the families a truncating *FGF16* mutation also segregated with heart disease. The importance of *Fgf16* in mouse heart development has previously been shown. We performed zebrafish functional knockdown of *fgf16* and showed heart oedema besides the expected recently described reduction of fin size.

Taken together, this thesis provides information about incidence and comorbidity of CULA and adds knowledge about phenotype and genetic mechanisms underlying radial polydactyly, X-linked recessive MF4 and the novel 17q22 microdeletion syndrome.